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Cell growth characteristics from angle- and polarization-resolved light scattering: Prospects for two-dimensional correlation analysis

Roxana M. Herran Cuspinera, Dennis K. Hore*

Department of Chemistry, University of Victoria, Victoria, British Columbia, V8W 3V6, Canada

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ABSTRACT

We highlight the potential of generalized two-dimensional correlation analysis for the fingerprinting of cell growth in solution monitored by light scattering, where the synchronous and asynchronous responses serve as a sensitive marker for the effect of growth conditions on the distribution of cell morphologies. The polarization of the scattered light varies according to the cell size distribution, and so the changes in the polarization over time are an excellent indicator of the dynamic growth conditions. However, direct comparison of the polarization-, time-, and angle-resolved signals between different experiments is hindered by the subtle changes in the data, and the inability to easily adapt models to account for these differences. Using Mie scattering simulations of different growth conditions, and some preliminary experimental data for a single set of conditions, we illustrate that correlation analysis provides rapid and sensitive qualitative markers of growth characteristics.

1. Introduction

Since its first demonstration by Noda in 1989 [1–5], generalized two-dimensional correlation spectroscopy (2DCOS) has gained increasing attention as a way of maximizing the information content of data-rich experiments [6,7]. The general idea is that, when a system is subject to an external perturbation (time, temperature, concentration, etc.), the measured signals will likely reveal their structural origins on account of their selective behaviours. For example, in the case of vibrational spectroscopy where 2DCOS has had the largest influence, spectra of even relatively small molecules are crowded with many overlapping bands. When a perturbation is applied, such as heating the sample, different bands will respond differently at each temperature. In the case of a mixture of proteins, various unfolding events result in changes to the infrared absorption spectra. Even though some of these changes may be subtle, linked events will result in spectral changes that are correlated. 2DCOS then provides a formal means by which such correlations may be revealed. This technique has been applied to many types of data sets, including those obtained from mid- and near-IR absorption [8,9], Raman scattering [10], nonlinear surface spectroscopy [11], electronic circular dichroism [12], and X-ray scattering experiments [13].

In all cases, there are generally two categories of motivation for performing the 2DCOS analysis. The first is to gain some quantitative structural insight, where the effect of the perturbation may be of direct interest (as when following the dynamics of system over time) or simply a means for more detailed spectroscopic study, such as the possibility for near-IR band assignment in a near-IR/mid-IR heterospectral correlation study [14]. The other possibility is that the 2DCOS synchronous and asynchronous maps are used to provide a rapid and sensitive means of fingerprinting, even for complex systems where individual molecules cannot be identified. A prime example has been the detection of counterfeit drugs [15,16]. Analysis by IR or Raman spectroscopy is rapid, and very sensitive to composition, but the differences between two samples are not readily revealed by the spectra themselves. There have been many examples in the case of traditional Chinese medicines where the 2DCOS maps obtained by heating different samples serve as fingerprints for the composition and formulation of the drugs, often containing a complex mixture of natural products [17–19].

In this work, we address a similar class of problem, characterizing the growth of bacteria in solution. It has been well demonstrated that polarized light scattering is a sensitive measure of cell morphology [20,21]. The polarization signatures as a function of scattering angle are so characteristic of the bacteria shape, that analysis of the data can lead to size distributions and aspect ratio information for rod-shaped cells [22]. This sensitivity has been

* Corresponding author.
E-mail address: dkhore@uvic.ca (D.K. Hore).

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utilized to distinguish different strains of the same species on the basis of the scattering profiles [23], to study the change in cell shape upon nutrient upshift [24], and to assess cell viability [25,26]. The measured signals are sufficiently sensitive to the growth conditions that they also reveal batch-to-batch variability when repeating experiments in the same lab [27]. We first demonstrate the proposed technique using data obtained from Mie scattering simulations to illustrate that 2DCOS is a useful method for fingerprinting cell growth. We then provide a demonstration of how experimental data may be collected and subject to 2DCOS to provide the markers.

2. Mie scattering simulations

It has been well-established that the angle- and polarization-resolved scattering profiles of cells may be reasonably approximated by Mie theory, the exact solution to Maxwell's equations for scattering by isotropic spheres of radius *r*. In addition to the size of the cells, the only other parameter required is the refractive index contrast, the ratio n_{rel} of the refractive indices of the cells to that of the medium.

Calculations were performed according to standard Mie theory [28–30]. The incident and scattered electric field may be described by a two-element vector E with complex-valued components parallel and perpendicular to the scattering plane. Mie theory provides the complex-valued elements $J_{11}(\theta; r, n_{rel})$ and $J_{22}(\theta; r, n_{rel})$ of the 2×2 Jones matrix J with $J_{12} = J_{21} = 0$ for spherically-symmetric scatterers. The input and output fields are therefore connected by

$$\mathbf{E}_{out} = \begin{bmatrix} E_x \\ E_y \end{bmatrix} = \frac{1}{ikR}\exp(-ikR + ikz)\cdot\mathbf{J}\cdot\mathbf{E}_{in} \tag{1}$$

where $\hat{z}$ is the propagation direction of the incident beam, $k = 2\pi/\lambda$ is the wavenumber and *R* is the distance from the scattering center to the detector. The Stokes vector describing the polarization of the scattered light may be obtained from the *x*- and *y*-components of E using

$$\mathbf{S} = \begin{bmatrix} S_0 \\ S_1 \\ S_2 \\ S_3 \end{bmatrix} = \begin{bmatrix} E_x E_x^* + E_y E_y^* \\ E_x E_x^* - E_y E_y^* \\ E_x E_y^* + E_y E_x^* \\ i\left(E_x E_y^* - E_y E_x^*\right) \end{bmatrix} \tag{2}$$

where the asterisk denotes the complex conjugate. In this representation, S_0 is the intensity of the light, S_1 is the difference in intensities between light polarized horizontally and light with vertical polarization, S_2 is the difference between linear states with azimuths 45° and 135°, and S_3 is a measure of the excess of right-hand circular over left-hand circular polarization states. The sample may be described in general terms by a 4×4 scattering matrix, the Mueller matrix. Studies of light scattering from cells have demonstrated that there are essentially 8 non-zero elements of **M**, of which 5 are unique considering that $M_{12} \approx M_{21}$, $M_{11} \approx M_{22}$, $M_{33} \approx M_{44}$, and $M_{34} \approx -M_{43}$ [31]. This simplified scattering matrix then appears as

$$\mathbf{M} \approx \begin{bmatrix} M_{11} & M_{12} & 0 & 0 \\ M_{12} & M_{11} & 0 & 0 \\ 0 & 0 & M_{33} & M_{34} \\ 0 & 0 & -M_{34} & M_{33} \end{bmatrix}. \tag{3}$$

The relationship between in the input and output polarization states is given by

$$\mathbf{S}_{out} = \frac{1}{k^2R^2}\mathbf{M}\cdot\mathbf{S}_{in}. \tag{4}$$

This provides $M_{12}(\theta; r, n_{rel})$, $M_{33}(\theta; r, n_{rel})$, and $M_{34}(\theta; r, n_{rel})$, each relative to M_{11}. For a collection of particles, elements of M are additive [32]. We therefore have a means of evaluating the response of a distribution of bacteria sizes

$$r(t) = N\exp\left[-\frac{[r - r_0(t)]^2}{2\sigma^2(t)}\right] \tag{5}$$

where *N* is a normalization constant defined such that

$$\int_0^\infty r(t)\mathrm{d}r = 1.$$

Here we allow the mean radius r_0 to evolve in time according to the empirical expression

$$r_0(t) = r_{min} + \frac{r_{max} - r_{min}}{1 + \exp[-k_r(t - t_r)]} \tag{6}$$

where in Case 1, we have considered a progression from $r_{min} = 520$ nm to $r_{max} = 560$ nm with a rate constant of $k_r = 0.4\ h^{-1}$, reaching the midpoint of the rise at $t_r = 15$ h. A similar sigmoidal evolution of the width of the bacterial population was used,

$$\sigma(t) = \sigma_{min} + \frac{\sigma_{max} - \sigma_{min}}{1 + \exp[-k_\sigma(t - t_\sigma)]} \tag{7}$$

with $\sigma_{min} = 15$ nm, $\sigma_{max} = 25$ nm, $k_\sigma = 0.2\ h^{-1}$, and $t_\sigma = 30$ h for Case 1. The resulting normalized M_{34} is shown in Fig. 1a, along with the 2DCOS synchronous (Fig. 1b) and asynchronous maps (Fig. 1c). This is known to be the most sensitive element of the scattering matrix for comparing small differences in populations [26,21,33]. Synchronous autopeaks are observed and expected at scattering angles near zero crossings, as those regions have the most possibility for changes in intensity when the population distribution shifts during cell growth. It is the pattern of cross peaks, however, that most uniquely characterizes this set of growth conditions. In this respect, the asynchronous map provides another level of fingerprinting, as it offers the possibility of distinguishing growth conditions that may not differ substantially in the synchronous maps.

The simulation was then repeated with a small difference in mean radius (Case 2) and width (Case 3) of the population distribution according to the parameters listed in Table 1. In Case 2 (Fig. 1d), the range of normalized matrix elements remains the same as in Case 1, with $-0.5 \leq M_{34} \leq 0.5$, however there is less change in $M_{34}(\theta)$ with time. This is immediately apparent in the visual pattern of the synchronous map (Fig. 1e), as the intensity and positions of auto- and cross peaks have changed. In the final example, the mean radius r_0 evolved in time with the same profile as in Case 1, but the evolution of the population width σ in the Gaussian distribution of radii was altered (see Table 1). The resulting M_{34} data (Fig. 1g) has a strong resemblance to that of Case 1, but the synchronous map (Fig. 1h) shows unique features that are characteristic of this particular set of conditions. All asynchronous correlation maps have appreciable intensity, and serve as a further qualitative indication of differences in the populations, and the manner in which the populations evolve in time. Using this simulated data set, we have demonstrated that 2DCOS of light scattering profiles can serve as a sensitive marker of cell growth conditions. We now provide an example of how such data may be obtained in